Provisional U.S. Patent Application No. 60/374,814
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ADDITIONAL INFORMATION

Enclosed please find a verified English language translation of Provisional U.S. Patent Application No. 60/374,814. Please file the translation simultaneously with the transfer of the international application to a U.S. phase to enjoy the benefit of the earlier filing date.

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	Yoshiaki MIYOTA et al	Conf.:	Unassigned
Appl. No.	Unassigned	Group:	Unassigned
Filed:		Examiner:	Unassigned
For:	FISH-FARMING SOLID FEED AND PROCESS FOR PRODUCING SAME		

VERIFICATION OF TRANSLATION

I, Yukio Uchida, hereby declare the following:

I am knowledgeable in Japanese and English. I have reviewed the specification of Provisional U.S. Patent Application No. 60/374,814 and believe the attached document to be an accurate translation thereof.

All statements made herein of my own knowledge are true and all statement made on information and belief are believed to be true. Further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: October 13, 2004

Yukio Uchida

Name of Document Specification

Title of the Invention

Fish-Farming Solid Feed and Process for Producing Same

Detailed Explanation of the Invention

[0001]

Industrial Field in Utilization of the Invention

This invention relates to a fish-farming solid feed containing an ascorbic acid derivative which exhibits an ascorbic acid activity and is stable, especially stable with time in the feed; and a process for producing the fish-farming solid feed.

[0002]

Prior Art

It is well known that deficiency or shortage of L-ascorbic acid in cultured fishes causes scurvy which occasionally causes death. For example, a plurality of abnormal fishes signed with scoliosis developed in many rainbow trout fishfarms in 1962. It was proved that these symptoms are caused by deficiency of L-ascorbic acid (Japan Fisheries Society, vol. 31, p 818-826). Deformed juvenile rainbow trout, red salmons and chum salmons were reported as caused by deficiency of ascorbic acid, in Annual Meeting of Japan Fisheries Society in 1967. Further, as examples of ascorbic acid deficiencies, there can be mentioned anorexia, slight exophthalmus, hemorrhage at a fin bottom, damage of gill cover and cervical damage in ayu sweet fish; feeding reduction, growth stop, scoliosis, abnormal pigmentation and high mortality rate in juvenile Japanese amberjack; and anorexia, growth reduction, fin hemorrhage and head hemorrhage in Japanese eel. Due to stress caused during culturing, a larger quantity of ascorbic acid is required for cultured fishes such as rainbow trout, red salmon, chum salmon, ayu sweet fish, cherry salmon, greater amberjack, Japanese amberjack, sea bream, common carp and Japanese eel, than the quantity required for wild fishes.

[0003]

Thus, vitamins including ascorbic acid are incorporated in a feed for juvenile fishes. However, ascorbic acid is an unstable water-soluble vitamin, and is easily decomposed within the feed. Ascorbic acid is extremely unstable especially in fish meal as a

protein source, and thus, it's vitamin C titer is greatly reduced by decomposition in a feed predominantly comprised of fish meal, such as feed for rainbow trout.

It is described in Japanese Unexamined Patent Publication No. H11-056256 that, when a fish meal having ascorbic acid incorporated therein is kneaded and extruded under high-pressure and high-temperature conditions by an extruder in the course of production of a fish-farming feed, ascorbic acid is greatly decomposed. In this patent publication, a technique is disclosed wherein an emulsion of water-soluble vitamins is applied to fish-farming feed pellets shaped from fish meal by an extruder. This technique is considered to avoid or minimize decomposition of water-soluble vitamins caused during kneading and extrusion by an extruder.

[0004]

To remedy the problem of decomposition of vitamin C, an attempt of incorporating vitamin C of a stabilized form, such as salts of L-ascorbate 2-phosphate, in a fish-farming solid feed has been proposed in Japanese Patent No. 2,943,785. However, the present inventors have found that, when the fish-farming solid feed is of a globular or columnar shape having a large diameter, or a large amount of vegetable oil and/or animal oil as a nutrient is incorporated in the fish-farming solid feed, vitamin C tends to be decomposed in an amount of at least 50% by mass based on the amount thereof added, even if it is of a stabilized form.

[0005]

Problems to Be Solved by the Invention

In view of the foregoing, a primary object of the present invention is to provide a fish-farming solid exhibiting high vitamin C titer which is reduced only to a minimum extent with the lapse of time. Especially even when the fish-farming solid feed has a large size and/or has incorporated therein a large amount of vegetable oil and/or animal oil, the fish-farming solid feed exhibits a high vitamin C titer which is reduced only to a minor extent with the lapse of time.

[0006]

Means for Solving the Problems

The present inventors made extensive research and found that decomposition of stabilized vitamin C including a salt of L-ascorbate

2-phosphate, as observed when a fish-farming solid feed containing the stabilized vitamin C is of a large size and/or the solid feed contains a large amount of vegetable oil and/or animal oil, occurs predominantly at the step of drying pellets as shaped at a kneading-and-shaping step using an extruder. Further, the inventors found that, when stabilized vitamin C is applied to dried pellets after the step of drying pellets, the substantial part of stabilized vitamin C, thus-lately applied, can be retained without any significant deterioration in a fish-farming solid feed. That is, the thus-applied stabilized vitamin C keeps its high vitamin C titer and exhibits high stability with time in the feed pellets. Based on these findings, the present invention has been completed.

[0007]

Thus, in accordance with the present invention, there are provided the following fish-farming solid feeds and processes for producing the fish-farming solid feeds.

[0008]

Fish-Farming Solid Feed

(1) A fish-farming solid characterized in that it contains stabilized vitamin C in an amount such that at least 50% by mass thereof, based on the total weight thereof contained in the solid feed, is present in a surface layer portion of the solid feed, spanning from the surface to a 1 mm depth.

(2) A fish-farming solid feed characterized in that it contains stabilized vitamin C in an amount such that the content thereof in a surface layer portion spanning from the surface to a 1 mm depth of the solid feed is at least 100 ppm by mass.

(3) The fish-farming solid feed as described in above (1) or (2), which further contains at least 10% by mass of a vegetable oil and/or an animal oil.

(4) The fish-farming solid feed as described in any one of above (1) to (2), wherein the content of stabilized vitamin C in the fish-farming solid feed is in the range of 100 to 5000 ppm by mass.

(5) The fish-farming solid feed as described in any one of above (1) to (4), wherein the solid feed has a water content of not larger than 10% by mass.

(6) The fish-farming solid feed as described in any one of above (1) to (5), which has a diameter of at least 11 mm.

(7) The fish-farming solid feed as described in any one of above (1) to (6), wherein the stabilized vitamin C is a salt of L-ascorbate 2-phosphate.

(8) The fish-farming solid feed as described in any one of above (1) to (7), which is used for feeding a fish or other aquatic animal selected from rainbow trout (Oncorhynchus mykiss), red salmon (sockeye salmon, Oncorhynchus nerka), chum salmon (keta salmon, Oncorhynchus keta), ayu sweet fish (plecoglossus altivelis), Biwa trout, cherry salmon (masu salmon, plecoglossus masou), greater amberjack (Seriola dumerili), Japanese amberjack (Seriola quinqueradiata), sea bream (porgy, Sparidac), common carp (Cyprinus carpio) and Japanese eel (Anguilla japonica).

[0009]

Process for Producing the Fish-Farming Solid Feed

(9) A process for producing a fish-farming solid feed as described in any one of above (1) to (8), characterized in that a fish farming feed material is kneaded and shaped into a shaped product; the shaped product is dried; and then, the dried shaped product is contacted with stabilized vitamin C having been dissolved, emulsified or dispersed in a liquid.

(10) The process for producing a fish-farming solid as described in above (9), wherein the shaped product is dried at a temperature of at least 110°C.

(11) The process for producing a fish-farming solid as described in above (9) or (10), wherein the shaped product is dried over a period of at least two hours.

[0010]

(12) The process for producing a fish-farming solid feed as described in any one of above (9) to (11), wherein the liquid in which stabilized vitamin C has been dissolved, emulsified or dispersed comprises a vegetable oil and/or an animal oil.

(13) The process for producing a fish-farming solid feed as described in any one of above (9) to (12), wherein the kneading of a fish farming feed material is carried out by a heated kneader.

(14) The process for producing a fish-farming solid feed as described in any one of above (9) to (13), wherein the dried shaped product is contacted with stabilized vitamin C having been dispersed in a liquid.

(15) The process for producing a fish-farming solid feed as described in any one of above (9) to (14), wherein the as-produced fish-farming solid feed contains, as measured immediately after the production thereof, at least 60% by mass of stabilized vitamin C based on the amount of stabilized vitamin C incorporated in the shaped product when the shaped product is contacted with the stabilized vitamin C-containing liquid.

[0011]

Modes for Carrying Out the Invention

The stabilized vitamin C includes, for example, salts of L-ascorbate 2-phosphate, and L-ascorbate 2-glucoside. The kind of stabilized vitamin C used in the present invention is not particularly limited provided that it exhibits higher stability with time in a fish-farming feed than that of natural vitamin C and it is capable of being converted to vitamin C within a living body. As preferable examples of the stabilized vitamin C, there can be mentioned magnesium, calcium, sodium and potassium salts of L-ascorbate 2-phosphate, and mixed metal salts thereof.

[0012]

The fish-farming solid feed of the present invention is characterized in that it contains stabilized vitamin C in an amount such that at least 50% by mass thereof, based on the total weight thereof contained in the solid feed, is present in a surface layer portion of the solid feed, spanning from the surface to a 1 mm depth; or, that the content of stabilized vitamin C in a surface layer portion spanning from the surface to a 1 mm depth of the solid feed is at least 100 ppm by mass.

The amount of stabilized vitamin C present in a surface layer portion spanning from the surface to a 1 mm depth of the sold feed is preferably at least 60% by mass, more preferably at least 65% by mass, based on the total weight thereof contained in the feed. The upper limit thereof is not particularly limited, but it is usually about 95% by mass. The content of stabilized vitamin C in a surface layer portion spanning from the surface to 1 mm depth of the solid feed is preferably at least 200 ppm by mass, more preferably at least 250 ppm by mass. The upper limit of the content in the surface layer portion is not particularly limited, but its upper limit is usually about 1.5% by mass.

[0013]

The fish-farming solid feed of the present invention containing stabilized vitamin C in a large proportion or content in the surface layer portion thereof is prepared preferably by a process wherein a fish-farming feed material is kneaded and shaped into a shaped product; the shaped product is dried; and then, the dried shaped product is contacted with stabilized vitamin C having been dissolved, emulsified or dispersed in a liquid.

To incorporate stabilized vitamin C in the fish-farming solid feed, it is preferable that the dried solid feed is contacted with stabilized vitamin C having been dissolved, emulsified or dispersed in a liquid, but the manner for the incorporation of stabilized vitamin C in the solid feed is not particularly limited. Further, any particular limitation is not imposed to the kind of liquid used, but a liquid containing water is not preferable. If a liquid containing water is used, drying is again required and thus the production process becomes long and complicated, and the re-drying may cause decomposition of applied stabilized vitamin C. It is preferable in view of stability that stabilized vitamin C is applied as a slurry, i.e., in a dispersed state. When such a slurry is used, stabilized vitamin C has greatly reduced chance of contacting with water, as compared with a solution or an emulsion, and its decomposition due to hydrolysis can be minimized.

[0014]

The liquid in which stabilized vitamin C is dispersed preferably includes vegetable oil and animal oil, which are conventionally added in fish-farming feeds. As specific examples of the vegetable oil and animal oil for fish-farming feeds, there can be mentioned soybean oil, rape-seed oil, corn oil, sesame oil, cotton-seed oil, safflower oil, sunflower oil, peanut oil, rice germ oil, wheat germ oil, camellia (Japanese rose, tsubaki) oil, palm oil, olive oil, jojoba oil, macadamia nut oil, avocado oil, caster-oil, linseed oil, beafsteak plant oil, eucalyptus oil, evening primrose oil, turtle oil, mink oil, lard, beef tallow and fish oil. These vegetable oils and animal oils may be used either alone or as a mixture of at least two thereof.

[0015]

In the case when stabilized vitamin C is applied as an emulsion, an emulsifier is used for the preparation thereof. The emulsifier

used includes, for example, sorbitan fatty acid esters, glycerin fatty acid esters, organic acid monoglycerides, propylene glycol fatty acid esters, diglycerides, sucrose fatty acid esters, polyglycerin fatty acid esters, lecithin, silicone surfactants and alkylene oxide-added surfactants. As specific examples of the emulsifier, there can be mentioned sorbitan monooleate, sorbitan distearate, polyoxyethylene(6 mols) sorbitan monostearate, glycerin monostearate, glycerin monolinolate, an esterified product of citric acid with glycerin monooleate, propylene glycol monostearate, glycerin dioleate, glycerin dilinolate, diglyceride obtained by ester interchange reaction of rape-seed oil with glycerin, diglyceride obtained by ester interchange reaction of safflower oil with glycerin, diglycerin distearate, diglycerin tristearate, hexaglycerin trioleate, hexaglycerin pentastearate, tetraglycerin condensed ricinolate, polyglycerin condensed ricinoleic acid ester, sucrose tri-, tetra- or penta-stearate, polyoxyethylene(5 mols) cetyl ether, polyoxyethylene(3 mols) nonylphenyl ether, polyoxyethylene(6 mols) stearyl ether, polyoxyethylene(5 mols) hardened castor-oil, polyoxyethylene(15 mols) hardened castor-oil, polyoxyethylene(20 mols) sorbitol tetraoleate, lecithin (e.g., Lecithin DX, Baycis LP-20, available from The Nisshin Oil Mills, Ltd.), dimethylsiloxane-methyl(polyoxyethylene(5 mols)-added) siloxane copolymer, sucrose fatty acid ester, polyglycerin fatty acid ester, lysolecithin, saponin, glycolipid, protein, protein decomposition products (other than gelatin), succrose stearic acid monoester, hexaglycerin oleic acid monoester, decaglycerin stearic acid monoester, enzyme-decomposed lecithin (e.g., Baycis LG-10K, Baycis LP-20E, available from The Nisshin Oil Mills, Ltd.), quillaiae saponin, soybean protein decomposition product, casein sodium, dimethylsiloxane-methyl(polyoxyethylene(60 mols)-added) siloxane copolymer, polyoxyethylene(25 mols) hardened castor-oil and polyoxyethylene(80 mols) hardened castor-oil.

[0016]

The contact of a dried shaped product of a fish-farming feed material with stabilized vitamin C is conducted in a manner such that usually about 100 to 5,000 ppm by mass, more preferably 100 to 2,000 ppm by mass of stabilized vitamin C, based on the weight of the dried shaped product, can be incorporated in the dried shaped

product. As examples of the procedure for incorporating the desired amount of stabilized vitamin C in the dried shaped product, there can be mentioned (i) a procedure wherein the dried shaped product is dipped in a stabilized vitamin C-containing liquid, (ii) a procedure wherein the dried shaped product is coated with a stabilized vitamin C-containing liquid, and (iii) a procedure wherein a stabilized vitamin C-containing liquid is sprayed or atomized on the dried shaped product.

[0017]

In one embodiment of the fish-farming solid feed of the present invention, stabilized vitamin C is predominantly contained in a surface layer portion of the solid feed. However, the bond strength of stabilized vitamin C to the solid feed is not particularly limited, provided that a predominant part of the deposited stabilized vitamin C is not separated during transportation from a producing district to a consumption district. An acceptable bonding strength can be advantageously obtained by the above-mentioned procedure of dipping the dried solid feed in a stabilized vitamin C-containing oil or spraying a stabilized vitamin C-containing oil to the dried solid feed.

The fish-farming solid feed of the present invention preferably contains at least 10% by mass, more preferably at least 20% by mass and especially preferably at least 25% by mass of vegetable oil and/or animal oil.

[0018]

To enhance the rate of absorption of vegetable oil and/or animal oil, the shaped product of fish-farming feed material is preferably dried to an extent such that the water content in the shaped product is reduced to 10% by mass or lower, more preferably 5% by mass or lower. If the water content in the shaped product is high, the rate of absorption of oil is low, and the efficiency of deposition of stabilized vitamin C is reduced. But, too low water content is not preferable because such a low water content is difficult to attain and a substantially long drying time is required with the result of partial decomposition of the active ingredient.

[0019]

The step of drying the shaped product as used in this specification means a step of drying the product as-shaped by a heated

kneader. The heated kneader used is not particularly limited, and includes, for example, an extruder. The drying can be carried out usually at a temperature of at least 110°C for at least 2 hours.

After the dried shaped product is contacted with stabilized vitamin C having been dissolved, emulsified or dispersed in a liquid, the shaped product having the liquid deposited thereon may be further dried by air drying or drying at a low temperature. This further drying is preferably carried out at a low temperature, for example, not higher than 90°C so as to avoid decomposition of stabilized vitamin C.

[0020]

Stabilized vitamin C is retained at a high ratio in the fish-farming solid feed of the present invention. That is, the solid feed contains at least 60%, preferably at least 80% and more preferably at least 90%, of stabilized vitamin C as measured immediately after the production thereof, based on the amount of stabilized vitamin C incorporated in the solid feed.

The shape of the solid feed is not particularly limited, and includes, for example, columnar shape, global shape and square pillar-shape. Especially a column-shaped solid feed called as dry pellet made by using an extruder is widely used. The fish-farming solid feed preferably has a diameter of at least 11 mm, more preferably at least 12 mm and especially preferably at least 13 mm.

[0021]

The ingredients contained in the fish-farming feed of the present invention, other than stabilized vitamin C, are not particularly limited, and can be those which are used in conventional fish-farming feeds, such as cereals, pulses, taros and potatoes, oil cake meals, brans, secondary products, animal feeds, vitamins, minerals, and compositions comprised of raw materials.

As specific examples of the cereals, pulses, taros and potatoes, there can be mentioned corn, milo (grain sorghum), wheat, barley, rye, oats, wheat flour, unhulled rice, millet seed, soybean, soybean flour and cassaba.

As specific examples of the oil cake meals, there can be mentioned soybean oil meal, skin-removed soybean oil meal, cotton seed oil meal, rape-seed oil meal, peanut oil meal, linseed oil meal, sesame oil, palm oil meal, sunflower oil, safflower oil, palm nut oil meal

and kapok oil meal.

As specific examples of the brans, there can be mentioned rice bran, white sake rice bran, defatted rice bran, wheat bran and barley-mixed bran.

As specific examples of the secondary products, there can be mentioned corn gluten feed, corn gluten meal, starch lees, molasses lees, soy strained lees, beer strained lees, beet pulp, bagasse, bean-curds refuse, malt root and orange juice strained lees.

As specific examples of the animal feed, there can be mentioned fish meal, white fish meal, fish solubles, fish solubles-adsorbed feed, meat meal, meat-and-bone meal, blood meal, feather meal, crab meal, shrimp meal, chrysalis oil meal, skim milk powder, dry whey and animal fat.

As specific examples of the minerals, there can be mentioned sodium chloride, potassium chloride, ferrous citrate, aluminum hydroxide, magnesium carbonate, calcium lactate, magnesium sulfate, sodium dihydrogenphosphate, ferric citrate, ferrous sulfate, potassium iodide and potassium iodate.

Further, the following ingredients can be mentioned. vegetable oils and fats such as soybean oil, rape-seed oil, corn oil and sesame oil; beer yeast, torula yeast, alfalfa meal, orange-peel, corn-tangle meal, tangle (kelp) meal, wakame sea-mustard meal, freshwater Chlorella, marine Chlorella, cellulose powder and carboxy-cellulose, and vitamin-mixed feeds thereof.

[0022]

Fishes to which the fish-farming solid feed of the present invention is given are not particularly limited, and include freshwater fishes, marine fishes and other aquatic animals such as crustaceans. As specific examples of the freshwater fishes and marine fishes, there can be mentioned rainbow trout (Oncorhynchus mykiss), red salmon (sockeye salmon, Oncorhynchus nerka), chum salmon (keta salmon, Oncorhynchus keta), ayu sweet fish (plecoglossus altivelis), Biwa trout, cherry salmon (masu salmon, plecoglossus masou), greater amberjack (Seriola dumerili), Japanese amberjack (Seriola quinqueradiata), sea bream (porgy, Sparidac), Japanese seabass (Lateolabrax japonicus), tiger puffer (ocellate puffer, Takifugu rubripes), bastard halibut (paralichthys olivaceus), goldfish, common carp (Cyprinus carpio) and Japanese eel (Anguilla japonica).

As specific examples of the crustaceans, there can be mentioned crustaceans, kuruma prawn (Japanese shrimp, Penaeus japonicus), black tiger prawn (giant tiger prawn, Penaeus monodon), river prawn (Macrobrachium spp.), swimming crab (horse crab, Portunus trituberculatus), Japanese spiny lobster (Panulirus japonicus), Japanese taisho prawn, western white shrimp, Chinese prawn (Penaeus chinensis), greasyback shrimp (Metapenaeus ensis), Japanese fan lobster (ahovel-nosed lobster, slipper lobster, Ibacus ciliatus), Japanese lobster (Metanephrops japonicus), Sakura shrimp (Sergia lucens), edible mantis shrimp (Oratosquilla oratoria), prawn, Japanese crayfish (Cambroides japonicus), lobster (Homarus americanus), tanner crab (Chionoecetes opilio), Alaskan king crab (Paralithodes camtschaticus) and helmet crab (Pagurus spp.).

[0023]

The invention will be described specifically by the following examples that by no means limit the scope of the invention. % in the examples is by mass.

[0024]

Example 1

Fish meal, wheat flour, soybean cake meal and fish oil were mixed together at a mass ratio of 60%, 15%, 5% and 20%, respectively, and, to enhance kneadability, water in an amount equal to the fish oil was added to the mixture. Then the mixture was kneaded thoroughly and extruded into a columnar extrudate having a diameter of about 14 mm. The columnar extrudate was pelletized and dried at 120°C for 3 hours until the water content became below 5% to give feed pellets.

Magnesium salt of L-ascorbate 2-phosphate (hereinafter abbreviated to "APM" when appropriate) was dispersed in fish oil to prepare an oily APM suspension having a concentration of 5,000 ppm by mass. The feed pellets were immersed in the oily APM suspension. After the immersion, the weight of feed pellets was increased by 5%. The 5% increase indicated that the amount of APM taken into the pellets was 250 ppm by mass. APM was extracted from the pellets and its amount was measured. The measured value was 234 ppm by mass.

The APM-containing feed pellets were stored at 40°C and, when one day, 3 days, 5 days and 10 days elapsed, APM was extracted and its amount was measured. The measured values (APM content in ppm) and retention (%) of APM content on the basis of 234 ppm as measured

immediately after the preparation. The results are shown in Table 1.

[0025]

Table 1

Retention of APM content

Number of days elapsed	1	3	5	10
Content of APM (ppm)	227	225	225	222
APM Retention (%)	97	96	96	95

[0026]

Method of Determining Content of APM in Solid Feed.

A solid feed containing a vitamin C derivative was thoroughly pulverized and placed in a mixed liquid (an aqueous 1% metaphosphoric acid solution/chloroform = 1/1). The mixture was shaken to extract the vitamin C derivative. An aqueous phase containing the extracted vitamin C derivative was analyzed by high performance liquid chromatography (HPLC) under the following conditions.

Column: "SHODEX™" J411 available from Showa Denko K.K.

Eluting solution: Mixed liquid of acetonitrile : 0.05M-KH_2PO_4 = 60 : 40 (w/w)

Flow rate: 1.0 ml/min.

Detection: UV with wavelength of 257 nm

[0027]

Example 2

A surface layer portion having a thickness of about 1 mm was shaven from the APM-containing solid feed pellets as made and before storage in Example 1. The surface layer portion was 0.53 g and the remaining core portion was 0.90 g. APM content in each portion was measured. The surface layer portion and the core portion contained APM in amounts of 510 ppm by mass and 75 ppm by mass, respectively.

[0028]

Example 3

Solid feed pellets were made by the same procedures as described in Example 1 except that each of sodium salt of L-ascorbate 2-phosphate (APS) and calcium salt of L-ascorbate 2-phosphate (APC) was separately used instead of APM.

The APS- or APC-containing feed pellets immediately after the preparation were tested for their APS or APC content. The APS content

was 230 ppm by mass and the APC content was 225 ppm by mass. These feed pellets were further tested for their APS- or APC-content retention (%) by the same methods as described in Example 1. The results are shown in Table 2 and Table 3, respectively. The methods of determining the content of APS and APC were the same as that for APM.

[0029]

Table 2

Retention of APS content

Number of days elapsed	1	3	5	10
Content of APS (ppm)	225	224	222	220
APS Retention (%)	98	97	97	96

[0030]

Table 3

Retention of APC content

Number of days elapsed	1	3	5	10
Content of APC (ppm)	220	219	215	215
APC Retention (%)	98	97	96	96

[0031]

Example 4

An APM-containing aqueous phase was prepared by dissolving 0.5 g of APM in 49.5 g of water. An oily phase was prepared by dissolving 5 g of hexaglycerin condensed ricinoleic acid ester ("Poem™" PR-300, available from Riken Vitamin K.K., HLB: 1.7) in 50 g of soybean oil at 80°C. While the aqueous phase was gradually added into the oily phase, the two phases were mixed together by a homomixer at 60°C and 6,000 rpm for 20 minutes to give an APM-containing emulsion.

A feed material mixture was kneaded, extruded and dried by the same procedures as described in Example 1 to give feed pellets. 0.5 g of the above-mentioned APM-containing emulsion was sprayed on 10 g of the feed pellets, and then the resulting feed pellets were air-dried. The content of APM in the as-made feed pellets was 228 ppm by mass.

[0032]

Example 5

An aqueous APM solution was prepared by dissolving 0.5 g of APM in 99.5 g of water. A feed material mixture was kneaded,

extruded and dried by the same procedures as described in Example 1 to give feed pellets. 0.5 g of the above-mentioned aqueous APM solution was sprayed on 10 g of the feed pellets, and then the resulting feed pellets were air-dried. The content of APM in the as-made feed pellets was 230 ppm by mass.

[0033]

Example 6

APM was dispersed in fish oil to prepare an oily APM suspension having a concentration of 10%. Using the oily APM suspension, APM-containing feed pellets were made by the same immersion procedures as described in Example 1. The weight increase of feed pellets as measured after the immersion in the oily APM suspension indicated that the amount of APM taken into the pellets was 5,000 ppm by mass. APM was extracted from the feed pellets and its amount was measured. The measured value was 4,900 ppm by mass.

The APM-containing feed pellets were stored at 40°C and, when one day, 3 days, 5 days and 10 days elapsed, APM was extracted and its amount was measured. The thus-measured retention (%) of APM content was 98%, 96%, 95% and 95% when one day, 3 days, 5 days and 10 days elapsed, respectively.

[0034]

Comparative Example 1

Solid feed pellets were made by the same procedures as described in Example 1 except that calcium salt of L-ascorbic acid (ASC) was used instead of APM.

The ASC-containing feed pellets were tested for their ASC content. The ASC content was 220 ppm by mass. These feed pellets were further tested for their ASC-content retention (%) at 40°C by the same methods as described in Example 1. The results are shown in Table 4.

[0035]

Table 4

Retention of ASC content

Number of days elapsed	1	3	5	10
Content of ASC (ppm)	180	110	66	11
ASC Retention (%)	82	50	30	5

[0036]

Method of Determining Content of ASC in Solid Feed

A solid feed containing a vitamin C derivative was thoroughly pulverized and placed in a mixed liquid (an aqueous 1% metaphosphoric acid solution/chloroform = 1/1). The mixture was shaken to extract the vitamin C derivative. An aqueous phase containing the extracted vitamin C derivative was analyzed by high performance liquid chromatography (HPLC) under the following conditions.

Column: "SHODEX™" J411 available from Showa Denko K.K.

Eluting solution: Mixed liquid of acetonitrile : 0.05M-KH_2PO_4 = 6 : 4 (v/v)

Temperature: 40°C

Flow rate: 1.0 ml/min.

Detection: UV with wavelength of 256 nm

[0037]

Comparative Example 2

Solid feed pellets were made by the same procedures as described in Example 1 except that APM was initially mixed together with fish meal, wheat flour, soybean cake meal and fish oil. The amount of APM added was 200 ppm by mass. The feed material mixture was kneaded, extruded and then dried at 120°C for 3 hours to give the solid feed pellets.

APM was extracted from the feed pellets and its content was measured. The measured content was 58 ppm by mass. Thus only 29% of the initially added APM remained.

A surface layer portion having a thickness of about 1 mm was shaven from the APM-containing solid feed pellets immediately after the preparation. The surface layer portion contained 50 ppm of APM and the remaining core portion contained 62 ppm of APM. The ratio of APM content in the core portion to that in the surface layer portion was 68 : 32.

[0038]

Effect of the Invention

The fish-farming solid feed of the present invention contains stabilized vitamin C at a high proportion or high concentration in the surface layer portion thereof. The vitamin C contained in the solid feed is stable and exhibits high vitamin C titer which is reduced only to a minimum extent with the lapse of time. Especially even when the stabilized vitamin C-incorporated fish-farming solid feed

has a large size, or has incorporated therein a large amount of vegetable oil and/or animal oil, the fish-farming solid feed exhibits a high vitamin C titer which is reduced only to a minor extent with the lapse of time.

Especially when the fish-farming solid feed is produced by a process wherein a fish farming feed material is kneaded and shaped into a shaped product; the shaped product is dried; and then, the dried shaped product is contacted with stabilized vitamin C having been dissolved, emulsified or dispersed in a liquid, the stability with time of vitamin C in the solid feed is remarkably enhanced.